(b) applying a magnetic field to the vessel so as to attract and immobilize the magnetic affinity particles;

(c) separating the unimmobilized remainder of the sample from the immobilized magnetic affinity particles;

(d) optionally, resuspending the magnetic affinity particles in a solution;

(e) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of detergent sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

66. (amended) A method for isolating a peptide, polypeptide or protein molecule from a sample in a vessel, comprising the steps of:

(a) combining the sample containing a peptide, polypeptide or protein molecule of interest with magnetic affinity particles suitable for binding said molecule, said magnetic affinity particles being insoluble in the sample;

(b) applying a magnetic field to the vessel so as to attract and immobilize the magnetic affinity particles;

(c) separating the unimmobilized remainder of the sample from the immobilized magnetic affinity particles;

(d) optionally, resuspending the magnetic affinity particles in a solution;

(e) optionally, eluting said molecule from the affinity particles, followed by separating the affinity particles from said eluted molecule;

wherein at least one of steps (a), (b), (c), (d) if present, and (e) if present is performed in the presence of detergent sufficient to reduce loss of particles during any separation step, in comparison to the same method performed in the absence of detergent.

REMARKS

Applicants have amended the claims to more clearly claim preferred methods of the invention for isolating a peptide, polypeptide, or protein molecule. Applicants have canceled Claims 1, 4, 9-12, 31, 33, 35, 40-43, without prejudice, which are directed to other embodiments, including methods for separating or isolating a nucleic acid molecule. Applicants reserve the right to pursue these claims in one or more continuating applications.

Applicants have amended Claims 2, 15, 34, 46, 64, and 66 to clearly cover methods of the invention for isolating a "peptide, polypeptide, or protein" molecule (plural forms in the case of Claims 15 and 46). Support for the amendments is found throughout the specification (see, e.g., p. 5, lines 19-20; p. 9, lines 15-19; p. 15, lines 22-23; and Examples 1-6, pp. 20-27). Accordingly, the amendments add no new matter.

Applicants have amended Claims 3, 13, 14, 16, 23-30, and 32 to remove dependency from canceled Claim 1. As amended, Claims 3, 13, 14, 16, 23-30, and 32 retain dependency from Claim 2. Accordingly, the amendments add no new matter.

Applicants have also amended Claims 44, 45, 47, and 54-63 to remove dependency from canceled Claim 33. As amended, Claims 44, 45, 47, and 54-63 retain dependency from Claim 34. Accordingly, the amendments add no new matter.

Applicants have also amended Claims 15 and 46 to clearly cover particularly preferred embodiments of the claimed invention for isolating peptides, polypeptides, or proteins using affinity particles in the presence of detergent. As noted above Applicants have added the terms "peptides" and "polypeptides" to a group of preferred possible binding partner molecules isolated by the claimed method. Applicants have also deleted recitation of the phrase "oligo-dT, nucleic acid polynucleotides complementary to a nucleic acid of interest" from the preferred list of possible affinity ligands that may coat an affinity particle and have deleted the terms "DNA, RNA, and small molecules" from the preferred list of possible binding partner molecules that may be isolated according to the claimed method. Accordingly, the amendments to Claims 15 and 46 add no new matter.

Entry of the above amendments is respectfully requested.

Claims Pending

Upon entry of the above amendments, the claims pending in this application are Claims 2, 3, 5-8, 13-30, 32, 34, 36-39, and 44-66. The amended claims are directed to methods of isolating peptide, polypeptide, and protein molecules using insoluble affinity particles and detergent at one or more steps in the methods of the invention.

The Invention

The claimed invention provides methods of isolating peptide, polypeptide, and protein molecules of interest comprising manipulating insoluble affinity particles in the presence of detergent. The presence of detergent at one or more steps in the isolation process reduces particle loss and facilitates manipulation of the affinity particles, e.g., collecting and separating from

solution, and results in increased yields of the peptide, polypeptide, or protein molecules of interest (see, e.g., Examples 1-6, at pp. 20-29 of the specification).

Rejections Under 35 U.S.C. § 102(e)

The Examiner rejected Claims 1-4, 9, 13-18, 19, 23, 24, 31-35, 44-50, 54, 55, and 62-66 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,942,391 (issued August 24, 1999, "Zhang"). The Examiner also rejected Claims 1-4, 9, 13-17, 20, 33-35, 44-46, 48, 49, and 64-66 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,466,577 (issued November 14, 1995, "Weisburg"). In particular, the Examiner was of the view that each of Zhang and Weisburg demonstrated each step in the claimed methods for isolating or separating a molecule and for specific embodiments wherein the molecule to be isolated or separated may be a nucleic acid. Applicants respectfully request reconsideration of these rejections in view of the amendments above and the remarks set forth below.

As noted above, Applicants have canceled various claims of the pending application, including Claims 1, 4, 9, 31, 33, and 35, thereby obviating both rejections as applied to these claims. In addition, Applicants have amended the remaining claims in the application to specifically cover Applicants' methods of isolating a peptide, polypeptide, or protein molecule comprising manipulating affinity particles in the presence of detergent, which advantageously reduces particle loss and increases yield.

The salient features of Zhang and Weisburg are reviewed below.

Zhang

Zhang describes methods *for detecting a target nucleic acid* from a pathogenic microorganism or from patients with genetic diseases or cancer (see, e.g., col. 3, lines 9-16; col. 5, line 61-col. 6, line 20 of Zhang). The methods of Zhang use multiple nucleic acid probes, including a "capture probe", which is attached to the surface of paramagnetic particles and which binds to a target nucleic acid molecule (see, e.g., col. 3, lines 52-59; Figure 1 of Zhang). One method described in Zhang is designated "ramification-extension amplification method" or "RAM" and employs two nucleic acid probes: a first probe (the "capture probe") linked to a paramagnetic particle, and a second probe (the "amplification probe") (see, e.g., col. 6, line 57-col. 7, line 5; Figures 1 and 7 of Zhang) used to amplify selected sequences of the target nucleic acid. Another method described in Zhang is a "hybridization signal amplification method" or "HSAM" system, which involves the use of an amplified number of signal probes that display a detectable signal indicating that a targeted nucleic acid has been bound by a target specific probe (see, e.g., col. 8, line 12-col. 9, line 6).

Weisburg

Weisburg describes nucleic acid probes *that hybridize to specific target sequences in the 16S ribosomal RNA* of *Borrelia* bacterial species, such as *B. bugdoferi*, the etiological agent of Lyme's Disease. Such probes are described by Weisburg as effective in the clinical diagnosis of Lyme's disease in tissue samples from humans and other animals (see, e.g., col. 3, lines 59-67). Weisburg also describes the use of such probes to detect *Borrelia* target nucleic acid in dot blots (see, e.g., Example 1, col. 6, line 66-col. 7, line 31 of Weisburg) and sandwich hybridization schemes where a "capture" probe binds a target sequence and a "detector" probe signals the binding of the target sequence (see, e.g., Example 2, col. 7, lines 35-62). Example 3 of Weisburg describes a sandwich hybridization protocol to diagnose Lyme's disease from blood in which the capture probe is linked to a magnetic particle (see, col. 8, lines 6-12).

The Examiner viewed Zhang and Weisburg as teaching Applicants' methods of isolating or separating a molecule of interest, and particularly nucleic acid molecules, that allegedly anticipate Applicants' claimed methods, which incorporate detergent to reduce loss of affinity particles. In addition, the Examiner stated that both Zhang and Weisburg taught the optional steps of eluting molecules from the affinity particles, followed by separating the affinity particles from the eluted molecule.

However, nowhere in Example 1of Zhang, referenced by the Examiner, is there a teaching that the target HIV-1 RNA molecule bound by the probes of Zhang was subsequently eluted from the probes. The section of Example 1 of Zhang cited by the Examiner describes how paramagnetic beads containing bound and amplified HIV nucleic acid sequences were washed (col. 27, lines 50-52) and subsequently used in a polymerase chain reaction (PCR) (col. 28, lines 1-17) to amplify the attached HIV sequence. The amplified nucleic acid molecules were detected by autoradiography (Fig. 3). Likewise, the section of Example 9 of Zhang cited by the Examiner describes the PCR amplification of a probe bound to a paramagnetic bead for detecting the target hepatitis C virus RNA molecules (see, col. 40, lines 19-20). In fact, the description specifically notes that after the step of ligating two probes, 10 µl of the ligation mixture "(including beads)" was transferred to a PCR mixture (see, col. 40, lines 19-20). Similarly, Example 3 in Weisburg, relied on by the Examiner, does not teach a method of eluting target *B. burgdorferi* nucleic acid molecules from probes attached to beads, and explicitly notes that the target molecules were detected by "spotting the beads on membrane and assaying by autoradiography" (col. 8, lines 23-25 of Weisburg). Clearly, Zhang and Weisburg do not teach methods to elute target molecules from probes.

More importantly, however, Applicants have amended the claims to specifically and clearly cover methods of the invention for isolating a peptide, polypeptide, or protein molecule of interest comprising manipulating affinity particles in the presence of detergent. The requirements for a reference to anticipate a claim are well established, as noted in the Manual of Patent Examining Procedure:

> **"TO ANTICIPATE A CLAIM, THE REFERENCE MUST TEACH EVERY ELEMENT OF THE CLAIM**
>
> 'A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.' *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). 'The identical invention must be shown in as complete detail as is contained in the . . . claim.' *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989)." (MPEP § 2131, emphasis in original).

Nowhere does Zhang or Weisburg describe Applicants' claimed methods for isolating a peptide, polypeptide, or protein comprising manipulating affinity particles and the advantage of using detergent in one or more steps of such methods to reduce loss of the affinity particles. Zhang and Weisburg describe methods of using specific types of nucleic acid molecules as probes to detect target nucleic acid molecules related to a disease. Clearly, neither Zhang nor Weisburg discloses "each and every element" of Applicants' claimed methods. Accordingly, Zhang and Weisburg are not references for anticipating Applicants' claimed methods under the patent law.

In view of the above comments and the amendments to the claims, Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 102(e).

Rejections Under 35 U.S.C. § 103(a)

The Examiner rejected Claims 1-4, 9, 13-17, 20, 25, 26, 33-35, 44-49, 56, 57, and 64-66 under 35 U.S.C. § 103(a) as obvious over Weisburg. The Examiner also rejected Claims 1-19, 23, 24, 31-50, 54, 55, and 62-66 as obvious over Zhang in view of U.S. Patent No. 5,646,016 (issued July 8, 1997, "McCoy"). Claims 1-4, 9, 13-19, 21, 23, 29-35, 44-50, 52, 54, 55, and 60-66 were rejected by the Examiner as obvious over Zhang in view of U.S. Patent 5,798,442 (issued August 25, 1998, "Gallant"). Finally, Claims 1-4, 9, 13-19, 22, 27-29, 31-35, 44-50, 52-55, and 58-66 were rejected by the Examiner as obvious over Zhang in view of U.S. Patent No. 4,009,213 (issued February 22, 1977, "Stein"). For the reasons discussed below, Applicants respectfully traverse the rejections.

As noted above, Applicants have canceled various claims of the application, including Claims 1, 4, 9, 31, 33, and 35 of the application, thereby obviating the Examiner's rejections as applied to any of the canceled claims. In addition, Applicants have amended the remaining claims in the application, as indicated above, to clearly claim Applicants' methods of isolating peptides, polypeptides, or proteins comprising manipulating affinity particles in the presence of detergent to advantageously reduce particle loss and enhance yields.

As discussed above, the primary references Zhang and Weisburg describe nucleic acid probes and their use to detect target nucleic acid molecules related to various diseases. Zhang describes methods employing multiple probes to detect a target nucleic acid molecule, either by amplifying copies of selected sequences in the target molecule ("RAM") or by using amplified numbers of signal probes to emit a detectable signal ("HSAM") to indicate that a target nucleic acid molecule has been bound by a probe for the target (see, e.g., col. 6, lines 4-8, Figs. 1 and 10 of Zhang). One type of probe is a "capture-amplification" probe described by Zhang which may be linked to a paramagnetic particle (see, e.g., col. 7, lines 25-30; Figs. 1 and 10 of Zhang). However, Zhang does not teach or suggest Applicants' claimed methods for isolating peptides, polypeptides, or proteins using affinity particles in the presence of detergent to reduce particle loss and to increase yields. Furthermore, Zhang does not provide a suggestion or motivation to be combined with any other reference cited by the Examiner to make Applicants' claimed methods of isolating peptides, polypeptides, or proteins. Accordingly, Zhang alone cannot render Applicants' claimed methods obvious under 35 U.S.C. § 103(a).

The Examiner also rejected claims of this application as obvious over Weisburg alone. As noted above, Weisburg describes nucleic acid probes that hybridize to specific sequences found in the 16S ribosomal RNA of *Borrelia* bacterial species, especially *B. burgdorferi*, which causes Lyme's Disease. Weisburg describes the use of such probes for the clinical diagnosis of Lyme's disease in humans and other animals (see, e.g., col. 3, lines 51-67). Such probes may be linked to a magnetic particle as described in Example 2 of Weisburg. However, nowhere does Weisburg contemplate, teach, or suggest Applicants' claimed methods for isolating peptides, polypeptides, or proteins using affinity particles which are exposed to detergent to reduce particle loss and increase yields.

Based on the above comments and the amendments to the claims, Applicants respectfully submit that it is clear that Weisburg, which is directed to methods of detecting specific nucleic acid molecules, does not render Applicants' claimed invention *prima facie* obvious. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejections based on Weisburg under 35 U.S.C. § 103(a).

The other references relied on by the Examiner fail to teach or suggest Applicants' claimed methods when combined. Considered together the references only provide an odd combination of disparate methods, not a disclosure of Applicants' claimed invention for isolating peptides, polypeptides, or proteins.

Zhang and McCoy

The secondary reference, McCoy, describes compositions and methods for enhancing expression of a protein of interest by constructing recombinant DNA molecules encoding a fusion protein comprising the protein of interest fused to thioredoxin (see, e.g., col. 3, lines 34-43 of McCoy). The fusion proteins of McCoy are further mutated to contain at least one "patch" of two or more metal chelating histidine residues, preferably in the thioredoxin portion, that enhances the metal binding ability of the fusion protein (see, e.g., col. 11, lines 9-58 of McCoy). The enhanced metal binding ability of the fusion protein is exploited during purification of the fusion protein by using a standard metal ion affinity matrix or resin (see, e.g., col. 16, lines 32-51). However, nowhere does McCoy teach or suggest Applicants' methods of isolating peptides, polypeptides, or proteins using affinity particles in the presence of detergent to reduce particle loss and enhance yields. Applicants' claimed methods are an improvement over the standard prior art methods as practiced in McCoy.

The Examiner appears to be of the view that McCoy provides motivation to be combined with Zhang:

> "It would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to substitute the affinity purification using histidine patch containing fusion proteins of McCoy et al in the affinity purification of Zhang et al since McCoy et al states, 'However, the present invention provides, inter alia, the modification of a fusion partner protein, e.g., thioredoxin, in such a way as to enable it to bind to a metal chelate affinity matrix, providing an additional convenient purification tool that can be used for fusion proteins. The technique is also applicable to other proteins, including other fusion partner proteins, and proteins which are not fusion protein constructs (column 3, lines 24-31)'. McCoy further provides motivation as he states, 'There is provided **another** novel method for increasing the production of soluble recombinant proteins (column 4, lines 22-24)'. An ordinary artisan would have been motivated by the express statement of McCoy to utilize the histidine patch containing fusion proteins of McCoy et al in the method of Zhang et al in order to achieve the express advantage of an improved affinity purification method with an additional

> convenient purification tool, as noted by McCoy et al, which can be used for increasing the production of soluble recombinant proteins and satisfactorily purifying them." (emphasis added, pp. 7-8, Office Action, Paper No. 10)

First, Applicants note that the statement at column 4, lines 22-24 of McCoy, mentioned by the Examiner (see, emphasis in above quote) is misquoted and misconstrued. The statement is taken from the "Summary of the Invention" and actually reads:

> "As yet **another aspect**, there is provided a novel method for increasing the production of soluble recombinant proteins."

Applicants respectfully submit that it is clear that the proper meaning of the above-quoted statement from McCoy is that it is an introduction of another embodiment of the McCoy invention and is **not** an express or implied teaching, suggestion, or contemplation of combining McCoy with other methods in the art. (See also the introduction to the paragraph in col. 4, line 22 of McCoy: "As yet another aspect, there is provided . . .".) Clearly, the actual statement from the text of McCoy is not under any logical construction an "express statement of McCoy to utilize the . . . proteins of McCoy et al in the method of Zhang et al" (p. 8, Office Action) to make Applicants' claimed invention obvious.

Furthermore, even if the methods of McCoy for producing certain fusion proteins are combined with the multi-probe nucleic acid detection methods of Zhang, the result is a collection of two completely different methods for two completely different molecules, i.e., detection of target nucleic acids (Zhang) and the expression and purification of certain mutant thioredoxin-fusion proteins (McCoy). A person of ordinary skill in the art who reads Zhang and then McCoy still does not arrive at Applicants' claimed methods of isolating peptides, polypeptides, or proteins in steps of manipulating affinity particles in the presence of detergent to reduce particle loss and enhance yields. Accordingly, the combination of Zhang and McCoy does not make out a *prima facie* case of obviousness to reject the claims.

Zhang and Gallant

Gallant describes a cysteine proteinase called apopain, which appears to play a key role in promoting apoptosis, and peptidyl derivative compounds that inhibit apopain (see, e.g., col. 1, lines 6-15; col. 10, lines 17-col. 14, line 56 of Gallant). Apopain cleaves the DNA repair enzyme poly (ADP-ribose) polymerase (PARP) in the early onset of apoptosis (see, e.g., col. 2, lines 36-47 of Gallant).

The Examiner relies on Gallant to provide a teaching of the use of the zwitterionic detergent CHAPS in an affinity purification method at col. 22, line 33-col. 23, line 27. The section of Gallant cited by the Examiner describes a purification scheme for apopain from the human monocytic leukemic cell line THP-1. In particular, the purification scheme involves applying a cytosolic fraction of THP-1 cells to a DEAE-5PW HPLC **column** that has been pre-equilibrated in a Tris/HCl buffer comprising 0.1% (w/v) CHAPS zwitterionic detergent. Proteins are then eluted from the **column** with a linear gradient of NaCl in Tris/HCl buffer also comprising 0.1% (w/v) CHAPS. Clearly, Gallant uses an HPLC **column** protocol that does not involve batch manipulations of affinity particles and the accompanying and critical problem of particle loss during such manipulations. Indeed, no such problem can even exist in the HPLC column protocol employed in Gallant because the chromatography particles remain packed in the HPLC column. In particular, a person of ordinary skill in the art would understand that the HPLC column method used in Gallant is distinctly different from Applicants' claimed methods, which clearly comprise the steps of collecting affinity particles from solution, separating affinity particles containing bound molecules from unbound components of a sample, optionally resuspending the particles in solution (as in a wash step), and optionally eluting and separating molecules of interest from the affinity particles to which they were bound. Each of the steps of Applicants' claimed methods is a manipulation of the particles that poses the very real opportunity to lose significant amounts of particles and thereby yield of the peptide, polypeptide, or protein of interest (see, e.g., Examples 1-6 of the instant application). In contrast, the anion exchange particles of the HPLC set up in Gallant are **never** manipulated during use, but are physically contained, and therefore, are never subjected to a manipulation (e.g., collecting, separating, washing) in which there is a risk of particle loss.

Clearly, Gallant does not practice the steps of Applicants' claimed methods and provides no recognition or insight into the problem addressed and solved by Applicants' invention.

Gallant is also devoid of any suggestion or motivation to be combined with Zhang to make Applicants' methods as required by law (see above). However, even if Zhang is combined with Gallant, the result is merely a disparate combination of two different methods used to achieve two different purposes: a method using a standard HPLC column apparatus to purify apopain proteinase and a multi-probe method of detecting target nucleic acid molecules. Clearly, Gallant adds nothing to advance the teachings of Zhang to arrive at Applicants' claimed methods of isolating peptides, polypeptides, or proteins in which affinity particles are manipulated in the presence of detergent to reduce particle loss and enhance yields. Accordingly, the combination of

Zhang and Gallant does not make out a *prima facie* case of obviousness to reject Applicants' claims.

Zhang and Stein

The method of Stein is an improvement of a known continuous process for separating mixtures of fatty alcohols that relies on manipulating fatty alcohol chemistry including converting fatty alcohol components in a mixture into different forms that have different melting points and dispersing and separating the subsequently formed liquid and solid forms of the converted fat compounds using aqueous wetting agent solutions (see, e.g., col. 3, line 55-col. 4, line 2 of Stein). Any of a variety of wetting agents may be used in the method of Stein (see, e.g., col. 5, line 59-col. 6, line 24 of Stein). The Examiner relies on Stein as a teaching for the use of the cationic detergent dodecyl trimethyl ammonium chloride (see, e.g., col. 6, lines 22-24). However, Stein does not use affinity particles for isolating peptides, polypeptides, or proteins in methods claimed by Applicants. Thus, Stein provides no recognition of the problem addressed by Applicants' claimed invention. Furthermore, Stein provides no suggestion or motivation to be combined with Zhang to make Applicants' claimed methods of isolating peptides, polypeptides, or proteins.

As with the other combinations of references set forth by the Examiner, even if Zhang is combined with Stein, the result is a confusing mixture of methods of using multiple nucleic acid probes to detect target nucleic acid sequences (Zhang) and a continuous process of separating fatty alcohols that relies on manipulating the chemistry and physical state of fat compounds (Stein). Even when Zhang and Stein are combined, the result provides no teaching or suggestion of Applicants' claimed methods. Clearly, Stein cannot cure the deficiencies of the primary reference Zhang to make Applicants' claimed methods obvious.

Based on the above comments and the amendments to the claims, Applicants submit that none of the references, alone or in the combinations set forth in the Office Action, renders Applicants' claims *prima facie* obvious. Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. § 103(a) are respectfully solicited.

In view of the amendments to the claims and all of the above comments, Applicants respectfully submit that all of the Examiner's rejections have now been avoided or overcome. Accordingly, the Examiner is respectfully requested to enter the amendments, withdraw the rejections, and pass Claims 2, 3, 5-8, 13-30, 32, 34, 36-39, and 44-66 to issue.

Respectfully submitted,

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CERTIFICATE OF MAILING

The undersigned hereby certifies that the items of correspondence referred to above are being deposited with the U.S. Postal Service as First Class mail under 37 C.F.R. §1.8, postage prepaid, in an envelope addressed to the Hon. Commissioner of Patents and Trademarks, Washington, D.C. 20231 on the date indicated below:

December 18, 2000
date of mailing and signature

Stephanie L. Leicht
Stephanie L. Leicht